HATCHING EGG SANITIZATION FOR PREVENTION OR REDUCTION OF HUMAN ENTEROPATHOGENS: A REVIEW

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Primary Audience: Researchers, Hatchery Managers, Hatching Egg Producers

SUMMARY

Hatching egg sanitization has been a somewhat controversial issue for more than 50 yr. Many in the hatching egg industry oppose wetting of fertile eggs. Nevertheless, research has been published on the problem of lowering bacterial contamination of hatching eggs without adversely affecting hatchability. As on-farm and hatchery Hazard Analysis Critical Control Point programs become a reality, more and more companies will become interested in how to best prevent human enteropathogen colonization in chicks. It is possible to effectively lower bacterial contamination on the eggs and in the hatching cabinet and thus reduce the level of human enteropathogens being placed in grow houses with the chicks. Information gleaned from both early and recent egg-sanitizing efforts can be useful in designing the best overall egg and hatchery sanitization program.

Key words: Disinfectant, hatchery, hatching eggs, *Salmonella*, sanitizer
2000 J. Appl. Poultry Res. 9:279–284

EARLY STUDIES ON EGG DISINFECTION

Because wet eggs are more likely to be penetrated by bacteria, the traditional wisdom among hatchery experts was to avoid wetting the egg at all costs, especially with liquid that was cooler than the egg [1]. However, research has shown that with careful control of disinfection procedures and parameters,

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hatching egg disinfection can be beneficial. By the 1940s poultry scientists were showing that wet sanitization of hatching eggs could be performed with no adverse effect on hatchability [2, 3]. Pritsker [2] showed that use of a disinfection solution warmer than the egg causes egg contents to expand rather than contract, so that penetration of the shell is avoided. This should have put to rest most concerns regarding wet egg disinfection. However, reports that negatively evaluate such procedures still appear [4].

Many studies have been conducted to examine different chemicals as hatching egg sanitizers. Sodium hydroxide in concentrations of 1 and 2% were found to be helpful in the control of *Salmonella* and other microorganisms without reducing hatchability [3, 5]. In the 1950s and 1960s several large studies compared various commercially available sanitizers as hatching egg disinfectants. Gordon *et al.* [6] concluded that when eggs were dipped in disinfectant solution within 1 hr of lay, no more cleaning was needed. They recommended two commercial products available at the time containing sodium pentachlorophenate or paraoctylpheoxyethyl benzyl diethyl ammonium chloride [6]. Lancaster *et al.* [7] found that the addition of a detergent component was helpful in the control of *Salmonella pullorum,* a chicken pathogen. Bierer *et al.* [8] agreed with earlier research by Pritsker [2] that formalin in 0.5% solution was an effective sanitizer, and showed its ability to kill *S. typhimurium*. However, because of the noxious characteristics of this chemical, Bierer *et al.* did not recommend its use. Nevertheless, research with the use of formaldehyde continued. Williams [9] found that application of formaldehyde by fumigation was very effective in lowering bacterial populations on the surface of hatching eggs. He recommended the use of formaldehyde on the farm, noting its efficacy, lack of penetration, and lack of detrimental effect on hatchability. As long as disinfectants were applied to the egg before or concurrent with challenge by *Salmonella,* penetration was prevented. However, as one would expect, *Salmonella* present on the egg prior to application of disinfectant can penetrate the shell deep enough to avoid direct contact with the chemical [10, 11].

Current Findings in the Area of Hatching Egg Disinfection

Egg sanitization continues to be a highly researched area, with many articles available detailing effectiveness of various chemicals and application methods. For the sake of brevity only a select group of more recent research efforts will be highlighted here.

Adverse health effects associated with the use of formaldehyde as a dip or fumigant have driven much of the current search for effective egg sanitizers. Patterson *et al.* [12] tested the use of chlorine dioxide applied to eggs as a foam in comparison to formaldehyde fumigation. Eggs were covered with a thick foam for 15 min before placement in the incubator and results compared to the old industry standard of formaldehyde fumigation of eggs in the incubator. Foam did not change hatchability compared to the untreated control group and lowered counts of inoculated *Escherichia coli* cells as effectively as formaldehyde fumigation.

Scott and Swetnam [13] compared a long list of sanitizers for "user friendliness" or hatchery personnel safety. Scott and Swetnam [13] claim the U.S. Department of Labor's maximum formaldehyde exposure level of 0.75 ppm is exceeded in traditional hatchery fumigation. A total of 23 sanitizers were rated based on the material safety data sheets provided by the manufacturer. Several sanitizers were deemed acceptable and all 23 were used in the second part of this three-part research effort. Scott and Swetnam [14] tested the chemicals' ability to lower microbial counts on the eggshell. With the exception of a product consisting primarily of quaternary ammonium compounds (Basic G + H), a chlorine dioxide product (Sanimist), ozone in solution and 7-day-old triple salt product (Virkon), all the chemicals lowered the total count to below detectable limits. It is important to note that the method for recovery in this study cannot detect organisms below the shell surface. Scott and Swetnam [14] used an outer shell rinse method to collect bacterial counts on eggs. Due to the porous nature of eggshell, a more sensitive method that detects cells trapped in the shell or membranes, such as a crush and rub method, would have been a

better choice [15]. Nevertheless, Scott and Swetnam's efforts [14] have significant merit, and they went on to test the effect of each sanitizer on the developing embryo in the egg. None of the chemicals were found to be clearly toxic to the embryo through the first 7 days of incubation [16]. However, some of the chemicals were noted to affect eggshell characteristics such as porosity and thus were not recommended. Several chemicals made it onto Scott and Swetnam's short list, one of which was hydrogen peroxide in a 0.7%, 1.4%, or 2.9% solution, providing a reduction from 122 colonies to below detectable limits [14].

Earlier, Sheldon and Brake found that 5% hydrogen peroxide was suitable as an egg disinfectant, eliminating culturable microorganisms, a 5.3 $\log_{10}$ CFU reduction, without adversely affecting hatchability [17]. Also in 1991, other researchers [18, 19] reported similar results with 1% hydrogen peroxide. Likewise, Padron [20] found that dipping eggs twice in 6% hydrogen peroxide was beneficial as a sanitizer, lowering bacterial counts on the membranes beneath the shell by 95%, and lessening salmonellae-positive eggs by 55%, without lowering hatchability. However, Padron's control group had only an 80% hatch of fertile eggs [20]. Research demonstrated that, in general, an immersion dip worked better than a spray [18, 19, 21]; however, a spray sanitizer machine would be more practical to apply disinfectants in a commercial setting.

Spray sanitizing machines are commercially available with integral water heaters that prevent the eggs from cooling and thus decrease bacterial penetration. Most machines are set up with a conveyor system that moves flats of eggs through a series of spray chambers, one with detergent to remove dirt and one with a sanitizing agent. Cox *et al.* [22] tested the use of a spray sanitizing machine on a commercial broiler breeder farm. Use of the sanitizing machine with a chlorine-based detergent and a quaternary ammonia-based sanitizer significantly lowered the total counts (a reduction of almost 4 $\log_{10}$ CFU) and coliform counts (a reduction of 1.4 $\log_{10}$ CFU) on eggs. The same machine was then tested using 10 different combinations of sanitizers. These combinations were applied to eggs that had been previously inoculated with *S. typhimurium*. Following sanitization, a highly sensitive detection procedure was used to determine presence or absence of *S. typhimurium*. Polyhexamethylenebiguanide hydrochloride (PHMB) proved to be the most efficacious chemical, resulting in an 85% reduction in the number of positive eggs compared to the water-washed control. Hydrogen peroxide also performed adequately, causing a 60% reduction [23].

Ability to kill microorganisms is not the only factor to be considered when deciding on a chemical egg sanitization regime. Shane and Faust [24] point out that cost of the chemical is an important consideration. However, in considering cost one must also consider the increased earnings potential associated with sanitizing fecally stained eggs and sending them to the hatchery, instead of to the breaker plant at a much lower price. Buhr *et al.* [25] found that in 1996 a producer of broiler eggs could make an extra $3,033 by sanitizing floor-slat eggs and sending them to the hatchery. This could result in paying for a commercial egg-washing machine within 2 yr. On the same set of eggs it was estimated that the hatchery realized an economic gain of $19,458 from the extra chicks.

Ultra violet (UV) light has been studied as a potential means to sanitize hatching eggs. Scott [26] tested the use of UV lights in hatching cabinets. Compared to formalin dipping, Scott found UV light to be ineffective as a pre-incubation treatment to lower total bacterial counts as detected by rinsing egg surfaces. When applied in the hatching cabinet, Scott found that UV light helped to prevent cross-contamination of pre-treated eggs from eggs that were not pre-treated. In general, Scott's findings do not point to UV as a promising technique for egg sanitization. However, Scott did not report the intensity of UV light that was used, nor did he attempt to increase intensity to test effectiveness. Berrang *et al.* [27] found that when eggs had been inoculated with a drop of *Salmonella* suspension on the surface, UV light at an intensity of 600 $\mu W/cm^2$ significantly lowered (but did not eliminate) the number of positive eggs. However, when eggs were inoculated with *Salmonella* in a smear of feces, UV light at intensities as high as 1600 $\mu W/cm^2$ was ineffective. Kuo *et al.* [28] examined the use of UV light to lower microbial counts from the eggshell surface. At an intensity of 620 $\mu W/cm^2$ significantly lower numbers of *S. typhimurium* cells, total counts,

and mold counts were recovered using a surface rinse technique. While UV light may hold some promise as an egg sanitizing agent, its usefulness is limited to clean eggs (without fecal staining). Because it is potentially harmful, UV light is most promising for application inside closed hatching cabinets as suggested by Scott [26].

Heat treatment of eggs has also been examined as a means to lower contamination. Due to the stress this would put on the embryo, heat treatments are suggested only for use on table eggs [29, 30, 31].

Sanitation Program to Control Hatching Egg Contamination with Human Enteropathogens

Increased attention to biosecurity on broiler breeder farms would greatly enhance the chances of producing a salmonellae-free egg. Careful attention to house disinfection between flocks and frequent effective cleaning of nest pad materials would lower the levels of microorganisms contacting the egg in the first few seconds post lay. Frequent egg collection followed immediately by an effective egg sanitization procedure with heated detergent and sanitizer would help to eliminate any salmonellae still on the surface of the egg. It is important to consider the chemistry of the detergent and sanitizer, being careful to avoid combinations that are incompatible (mixing to produce dangerous products) or that may counteract each other. Ideally, temperature and/or humidity would be controlled during transport, storage, and setting in the incubators to prevent wetting the eggs with condensation, which could facilitate re-contamination.

Even ideal collection, disinfection, and transportation protocols would not eliminate bacterial pathogens if an egg had been subjected to trans-ovarian contamination or if horizontal contamination penetrated beneath the shell very quickly. However, once an egg is in the incubator, the effects of cross-contamination can still be controlled. Whistler and Sheldon [32] found that the application of an ozone mist at 3% by weight as a disinfectant in the setter can lower microbial counts. Perhaps more important is the application of a fog or mist disinfectant in the hatcher, where the greatest potential for cross-contamination among many chicks exists. Bailey *et al.* [33] found that a fine mist of 2.5% hydrogen peroxide during the last 3 days of incubation significantly reduced the likelihood of *Salmonella* cross-contamination from *in ovo*-inoculated chicks to uninoculated chicks. Hydrogen peroxide applied in this manner did not lower hatchability, which was maintained at 95% hatch of transfer.

Egg and hatchery sanitation will be a critical part of producing *Salmonella*-free poultry. For many years commercial poultry feed and feed ingredients were believed to be the primary contributors to salmonellae contamination of poultry. Most poultry producers felt that if salmonellae could be eliminated from the feed, the problem would be solved. Unfortunately, the situation is much more complex. Various studies have shown that *Salmonella* serotypes found on the final product (fully processed broiler carcasses) can originate from sources other than feed, such as hatcheries and breeder flocks [34, 35, 36]. Regardless of the source, protecting the newly hatched chick from exposure to salmonellae is extremely critical because the young animal lacks mature gut microflora and is highly susceptible to intestinal colonization by *Salmonella*. Research has shown that very low levels of *Salmonella* can colonize the intestinal tract of young broiler chicks. Such exposure can come through an assortment of body openings, including the mouth, nasal passages, eye, and cloaca [37]. Salmonellae originating from the breeder flocks have resulted in the establishment of reservoirs in the commercial broiler and breeder hatcheries [38, 39]. These reservoirs will continue to exist until salmonellae have been eliminated from breeder flocks or the hatching eggs by an effective chemical treatment. Research to date suggests that hatching eggs can be disinfected if an effective chemical is applied as soon as possible after exposure of the egg to *Salmonella* [40, 41].

Even after eliminating or minimizing the effect of breeder flock and hatchery contamination, the newly hatched chick must be protected from environmental sources of salmonellae in the grow house. One effective approach to prevent the intestinal colonization of live poultry by salmonellae

is competitive exclusion (CE), which was first described by Nurmi and Rantal [42]. However, poor egg and hatchery sanitation cannot be remedied by applying CE as the chicks leave the hatchery. Salmonellae contamination in the hatchery has been shown to limit the effectiveness of CE [43]. Therefore, the combination of eliminating or dramatically reducing salmonellae from hatching eggs and hatcheries, followed by treatment of new hatchlings with an effective CE culture before exposure to environmental salmonellae, presents a realistic opportunity to produce a salmonellae-free broiler.

Conclusions and Applications

1. Hatching eggs can be sanitized without affecting hatchability.
2. Spray sanitization with certain chemicals can lower the population of salmonellae on hatching eggs.
3. A combination of egg sanitization, application of sanitizer during pip, and treatment with an efficacious competitive exclusion product offers the best chance for a salmonellae-free chick.

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